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# PAL RTC Hardware Configuration

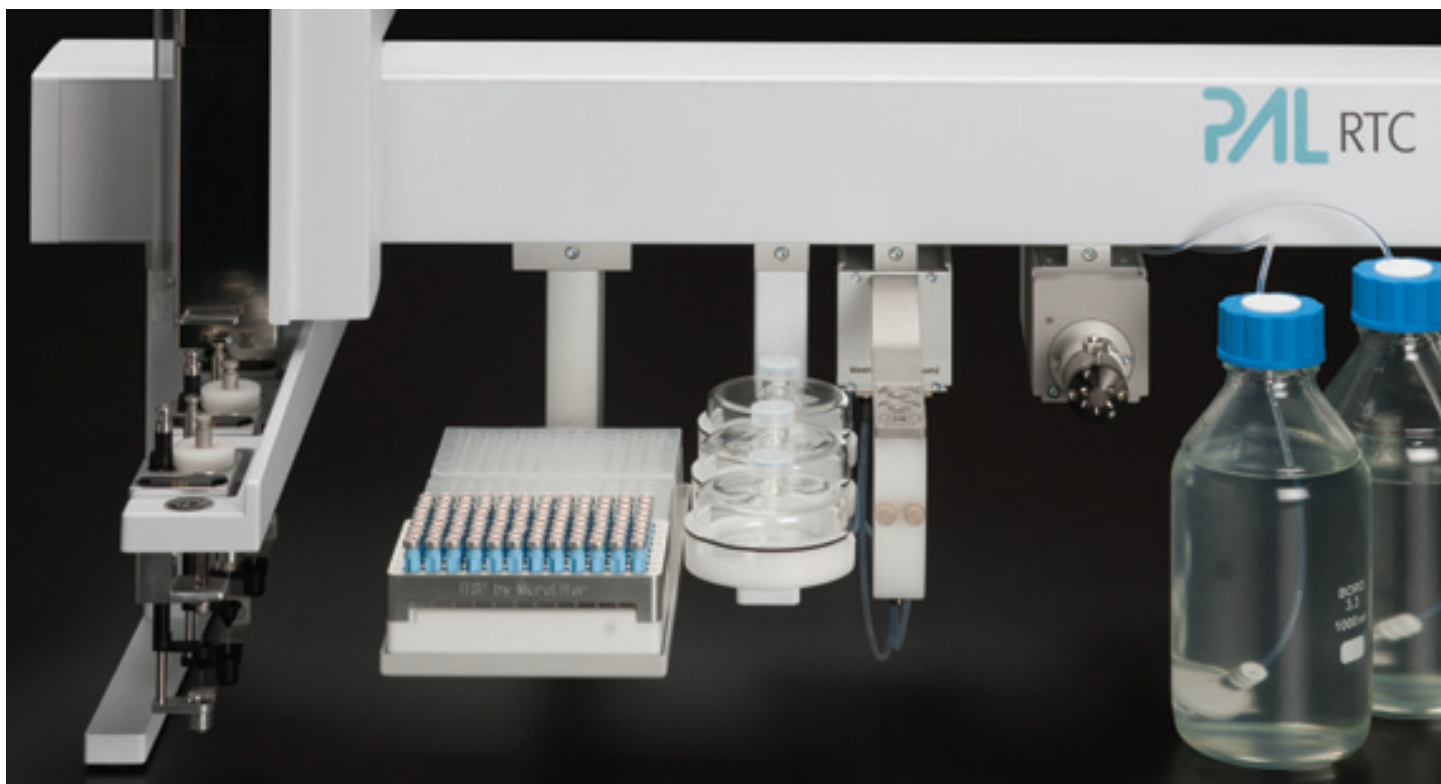
This guide offers installers detailed instructions for installing hardware associated with ITSP® solutions for on-line sample preparation.



## Introduction

This set-up guide details specific hardware configuration for installers and users who wish to use an ITSP solution for an automated on-line Solid Phase Extraction clean-up using the PAL RTC. In general there are but a few concepts that must be understood to apply ITSP technology. The most basic being that one must elute into a recovery vial or 96-well microplate then return to this same location to aspirate the eluate for injection onto the analytical instrument, whether it be GC/MS, GC/MS/MS, LC/MS or LC/MS/MS. To facilitate this task one must visualize two objects in the same location and define these in the hardware configuration phase of the PAL RTC installation. This guide will walk you through these steps and make it easier to quickly move to the chemistry phase of implementing this robust solution for on-line automation.

For programming your PAL RTC System for an ITSP solution refer to the PAL System, PAL Sample Control (PSC) User Manual or PAL Script User Manual for popular chromatography operating systems. Also see sample methods loaded within the software for basic templates for single-elution Solid Phase Extraction (SPE), filtration and ITSP SPE clean-up of QuEChERS extractions. These templates can be used to build specific modifications related to your application and your instrument's sensitivity.



This photograph shows an assembled PAL RTC hardware configuration for an ITSP-LC/MS/MS instrument. The set-up is the same for GC instrumentation as it relates to ITSP hardware requirements. Other more familiar items such as elimination of the valve injection port assembly and attachment hardware instead of freestanding legs specifically designed for fitment onto each manufacturer's GC instrument are all that changes with respect to GC installations. Of course, syringe tools specific for both ITSP and injection are key decisions that must also be made. All of the PAL RTC firmware objects specific for the ITSP solution hardware kit (ITSP product number: HW3-KIT) are embedded in the firmware of the RTC and can be accessed by computer or by the remote terminal via  
Set-Up > PAL modules and do not require installation by the installer.

The ITSP product number: HW3-TRAY is a PAL RTC 3-position tray holder that is a specific requirement for ITSP. It has a hole drilled in the base of the tray in Slot 3 for attaching a waste receptacle hose bib through the tray and routed to a waste container of sufficient size to handle byproducts produced by the extraction processes. It is trained and configured as two individual tray holder objects in the same physical location as Tray Holder 1 and Tray Plate 1.

If the sample run's quantity of samples exceeds the capacity of the ITSP Hardware Kit additional workstations can be added as Tray Holder 2 and Tray Plate 2, etc.

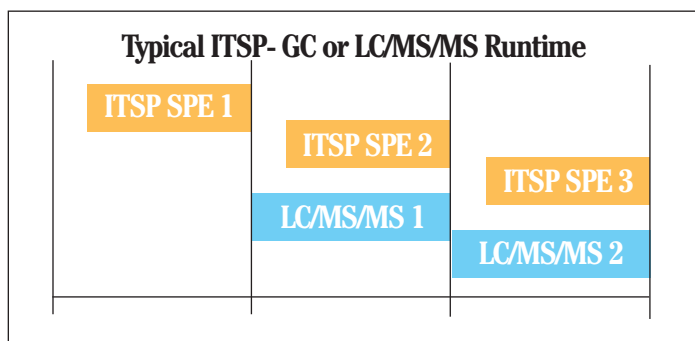
Take a moment before continuing the installation to review the fundamental mechanics of ITSP cartridges and operation of the PAL RTC's integration into the analytical instrument's operating system.

## The mechanics of ITSP's patented cartridges for solid phase extraction

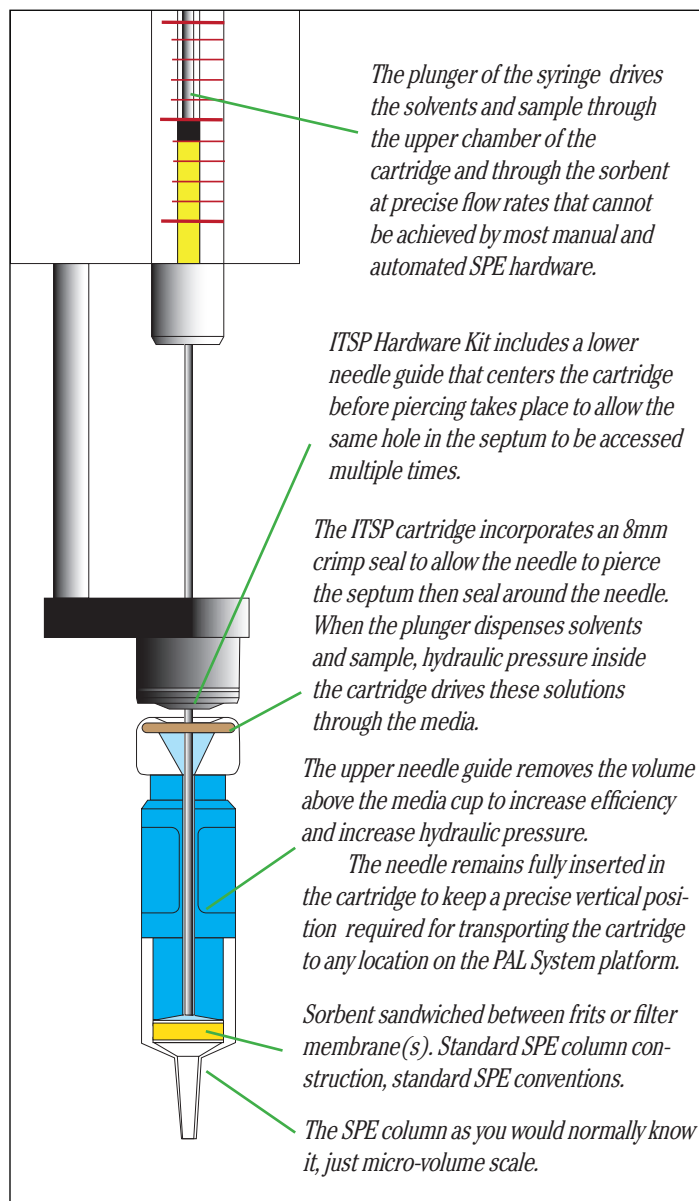
ITSP Solutions' patented cartridge for automated SPE uses precise control of flow rates and volumes for solvents and samples through the sorbent by the PAL System while the LC/MS/MS or GC/MS/MS is analyzing a sample in parallel.

The PAL RTC System uses ordinary autosampler tray holders, trays, syringes and solvent reservoirs to which we adapt our single position solution to automate SPE, the gold-standard for sample preparation for Liquid and Gas Chromatography.

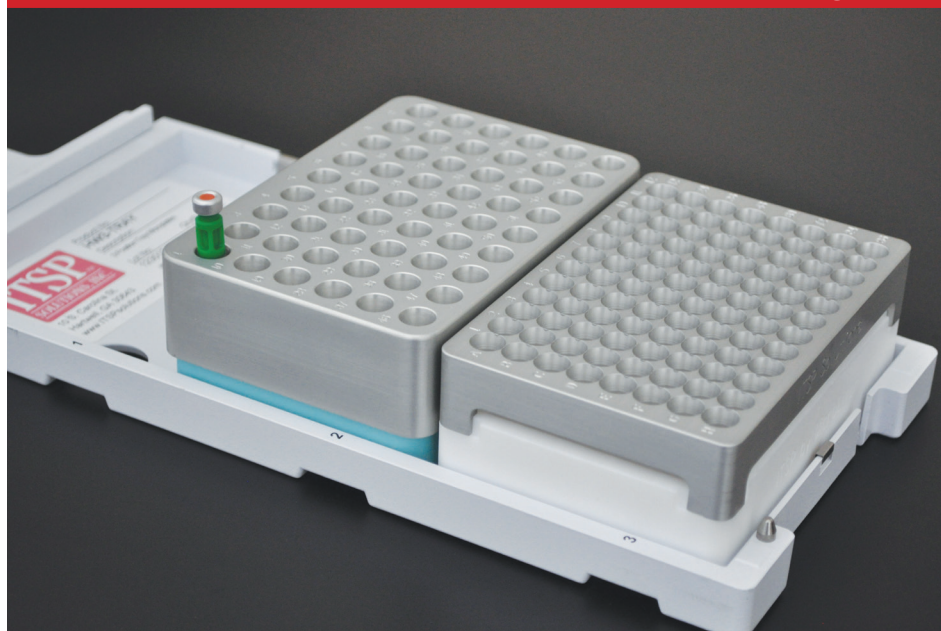
By aspirating a solvent or sample, then penetrating the septum on the ITSP cartridge, the syringe plunger can pass both through the sorbent bed at higher precision than achievable by ordinary manual and automated vacuum-based and pneumatic systems (none of which have flow control). The illustration in the next column details the construction of the ITSP cartridge.



An ITSP solution uses the analytical instrument's look-ahead feature embedded into the analytical instrument's operating software to prepare samples while the instrument is analyzing the prior sample. Notice how ITSP elutes the sample just-in-time for injection.



## PAL RTC Hardware Kit for ITSP solutions using autosampler vials



### PAL3 RTC ITSP Autosampler Vial Tray Setup;

- 3 position tray holder with an ITSP cartridge prep location in the front slot,
- 2mL vial tray to receive the ITSP elution in the middle tray slot with HW-54P Vial Lock. This tray holds 54, 2mL vials with starburst septa for penetration of the septa by ITSP cartridges.
- Client sample location in the rear slot to hold a VT54, 2mL vial rack with closed septa vials.

Photograph 1 shows the ITSP Solutions, Inc. PAL RTC Hardware Kit, HW3-KIT. The kit consists of the modified 3-position tray holder, ITSP waste receptacle, a sample 96-position prep plate, and lower needle guide for a PAL RTC Tool. The HW3-NG, ITSP-modified PAL RTC lower needle guide, replaces the existing lower needle guide on whichever PAL RTC tool will be used for ITSP steps in the method. It has been modified to mate exactly with the crown on the ITSP cartridge seal in order to enhance robust PAL operation while executing ITSP activities.

Photographs 2-4 below show the various components needed to set up the PAL ITSP tray holder for a vial-based ITSP PAL RTC method. **IMPORTANT:** Please note that it is very important for robust operation that the ITSP cartridge tray (Tray Plate 1: Slot 3 – Tray Type ITSP96) be carefully and precisely trained so that the needle hits the ITSP cartridge seal septa exactly in the center of the septa.



ITSP Solutions Hardware Kit, HW3-KIT for PAL RTC includes:

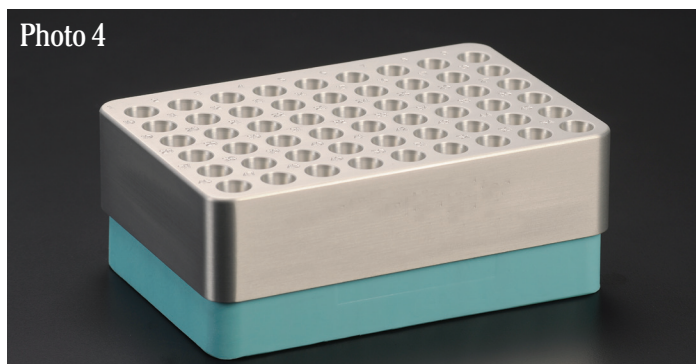
- HW3-TRAY - 3-position TrayHolder,
- HW3-NG - ITSP needle guide,
- HW96P - 96-position aluminum ITSP cartridge tray, &
- HW-WP - Delrin ITSP waste receptacle with waste tube connector



CTC Analytics VT54 vial tray shown loaded with 2mL vials. ITSP elution trays utilize snap caps with starburst pre-cut PTFE/Silicone septa (inset) available from almost all consumables supply companies. When eluting in a closed vial is desired, ITSP can easily pierce these septa when topped with the HW-54P Vial Lock and retract from the vial without binding.



ITSP Solutions product no.: HW-54P, 54-position ITSP Vial Lock, for securing 2mL vials with pre-cut starburst septa while eluting with ITSP.



ITSP Solutions product no.: HW-54P, ITSP Vial Lock shown on top of the VT54 vial tray loaded with 2mL vials. This tray set includes the CTC Analytics VT54 vial tray and samples of snap caps with pre-cut starburst septa.

## PAL RTC trays for ITSP SPE cleanup methods

Tray	Tray Type	Description	Rack Teaching
Tray Holder 1:Slot1	VT54* or DWP96	Sample tray with 12x32mm vials or 96-well microplate with cover	VT54 vial tray with 2mL vials or DWP96 96-well microplate
Tray Holder 1:Slot2	HW-54P or DWP96	Eluate tray (vial rack or microplate) holds samples for injection, after being cleaned up using ITSP	Top of HW-54P tray without ITSP cartridges or 96-well microplate without cover
Tray Holder 1:Slot3	Empty – Not used		
Tray Plate 1:Slot1	Empty – Not used		
Tray Plate 1:Slot2	VT54ITSP or DWP96ITSP	ITSP elution tray	ITSP cartridges manually placed in corner positions 1, 47 and 54 of HW-54P tray or 1, 88, 96 of uncovered 96-well microplate
Tray Plate 1:Slot3	ITSP96	ITSP cartridge prep tray	Top of HW-96P tray loaded with ITSP cartridges
Solvent Module1	Solvent module	3 100mL vials for ITSP SPE solvents	
Waste 1	Waste location	Disposal beaker for discarding spent ITSP cartridges	Trained over an empty beaker large enough to hold approximately 100 discarded ITSP cartridges.

\* PAL RTC Trays for ITSP SPE or filtration cleanup methods are referenced by their definition in the firmware of the PAL RTC. Actual CTC Analytics product numbers vary from those listed above.

**Tray Holder 1 and Tray Plate 1** are two logical tray holders trained in the same location – they are the same physical tray holder. Tray Plate 1 holds ITSP cartridges for conditioning in slot 3 and for elution in slot 2, Tray Holder 1 holds 2mL sample vials or a 96-well microplate in slot 1 and eluate vials beneath HW-54P or microplate in slot 2.

**Tray Holder 1: Slot1** can be assigned any tray type valid for sample trays. Use the chart above to designate either a vial or microplate specific for your samples. It is used in an ITSP PSC method or PAL script to hold the original raw sample, e.g. QuEChERS extracts or prepared biological matrix.

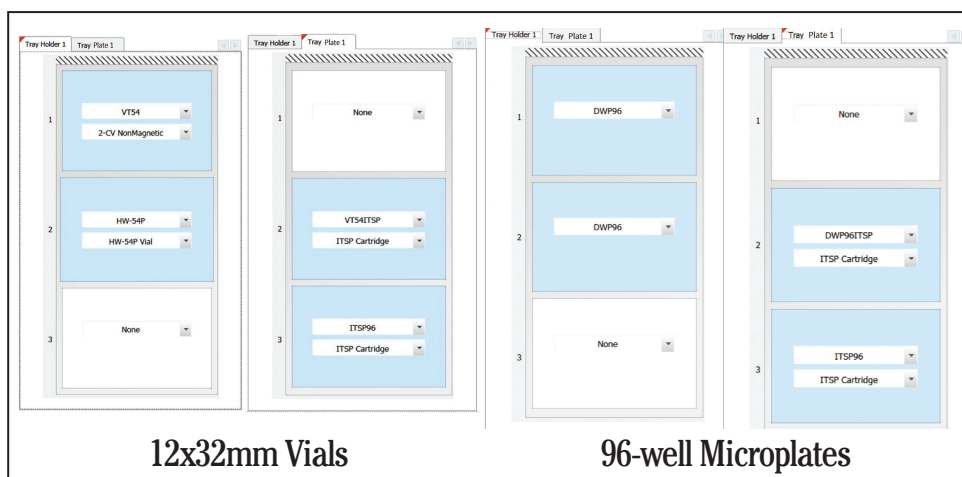
**Tray Holder 1: Slot2** is assigned a tray type defined above, and is configured and trained without any ITSP cartridges, just the HW-54P sitting on top of a VT54 tray (in the same physical slot 2 of the TrayHolder as the VT54ITSP tray) filled with 2mL vials capped with starburst pre-slit septa or an open-well 96-well microplate. It is used in an ITSP PSC method or PAL3 script to add dilution solvents after ITSP elution or to aspirate an aliquot for injection from the autosampler vial or microplate.

**Tray Holder 1: Slot3** is empty, not used.

**Tray Plate 1: Slot1** is empty, not used.

**Tray Plate 1: Slot2** is assigned a tray type configured with ITSP Cartridge and is configured and trained to recognize the ‘Z’ height position for the ITSP elution in to the vials or microplate below by manually placing ITSP cartridges in the corner positions of the defined tray type specified above. It is used in an ITSP PSC method or PAL script to elute to a sealed vial or microplate in the ITSP cleanup method.

**Tray Plate 1: Slot3** is assigned an ITSP96 Tray Type configured with ITSP Cartridge and is configured and trained with ITSP cartridges in all positions of the HW-96P tray mounted on top of the HW-WR ITSP waste receptacle. It is used to hold ITSP cartridges and to condition (“prep”) ITSP cartridges over waste.



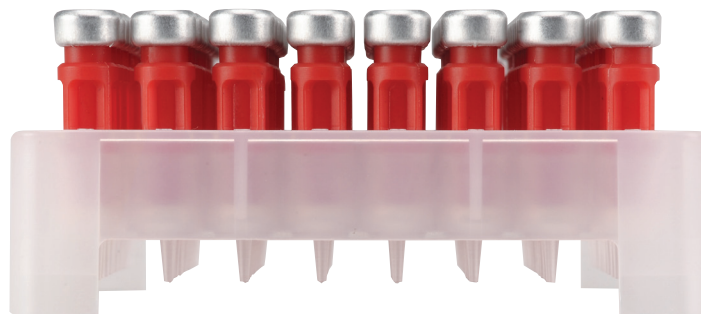
Screen shot of the depiction of the two tray holders in the set-up screen, Tray Holder 1 'as is' and Tray Plate 1 as a 'virtual' Tray Holder in the same position.



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US Patents: 6,859,615 & 7,001,774 • European Patents: EP 1 174 701 • Canadian Patent: 2,316,648